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Estimation of Quercetin in *Acacia nilotica* Linn (Flowers) using HPTLC

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ABSTRACT

A simple and fast method was developed for quantitative determination of quercetin in flower of *Acacia nilotica* using high performance thin layer chromatography (HPTLC). The separation was performed on TLC aluminum plates precoated with silica gel 60 $F_{254.}$ Good separation was achieved in the mobile phase of chloroform : methanol : water (7: 3: 0.5 v/v) and densitometry determination was carried out at 280 nm in absorbance mode. The linear regression data for the calibration plots showed a good linear relationship with r = 0.9999 for quercetin. Accuracy of the method was checked by recovery of 102% for quercetin. The present method is being reported first time and may be used for routine quality control of the flower of *A.nilotica.*

Key words: HPTLC, *Acacia nilotica*, Quercetin

INTRODUCTION

Since ancient times, traditional herbal medicines have been extensively used to prevent and cure human diseases because of low price, easy accessibility by the local people and most importantly low toxicity. A large number of compounds like vitamins, amino acids, carotenoids, terpenoids, alkaloids, tannins and phenolic compounds are responsible for activities of traditional herbal medicines. Among them, phenolic compounds have been reported to exhibit wide range of biological effects [1, 2]. So the quality control and quantitative analysis of phenolic compounds in traditional herbal medicines have become a necessary undertaking. *A. nilotica* an important medicinal plant was selected to develop a better method for analysis of its phenolic compounds by high performance thin layer chromatography (HPTLC).

A.nilotica (Fabaceae) locally known as Karuvael , is common in drier parts of India, occurring mainly at an altitude of 900m.The bark, gum, leaf, seed, pod of *A.nilotica* are important crude source of drugs in traditional medicines [3-7] .The flowers are golden yellow ,fragrant, crowded in long stalked globose heads, forming axillary clusters of 2 to 5 heads. Flowers of *A.nilotica* are a rich source of kaempferol-3-glucoside , isoquercetin and leucocyanidin [8]. Regarding phenolic compounds, a limited number of reports disclose the presence of quercetin in flowers of *A.nilotica* but detail investigation not done [8]. These phenolic compounds posses a broad range of physiological activities including antioxidant, anti inflammatory and antibacterial activity [9-12] However, no report is available on the quantitative assay of these phenolic compounds in *A.nilotica* .Keeping this in view, the present study was carried out with an objective of developing a simple and fast method for simultaneous determination and quantification of quercetin in methanolic extract of flowers of *A.nilotica* using HPTLC.

MATERIALS AND METHOD

Reagent and Materials

The flowers of *A.nilotica* were collected from Thanjavur, Tamilnadu. Shade dried and coarsely powdered. Reagents, Standard Quercetin was purchased from M/s Sigma chemicals. Aluminum plates precoated with silica gel 60 F_{254} of 0.2mm thickness (E. Merck, Darmstadt, Germany) were used without pretreatment. All chemicals and solvents used were analytical and HPLC grade (E.Merck, Mumbai, India).

Analytical procedure

Chromatographic conditions

Chromatography was performed on a silica gel HPTLC 60 F_{254} 20X20 cm with 0.2mm thickness plate. Sample and standards were applied to the plate as 6 mm wide bands with an automatic TLC applicator Linomat V with N_2 flow (CAMAG, Switzerland), 10 mm from the bottom and 13 mm space between two bands were identical for all the analyses performed.

Detection of quercetin

The HPTLC Plates were developed using a CAMAG twin trough glass tank which was presaturated with the mobile phase chloroform-methanol-water (7:3:0.5) for 1 hour and each plate was developed to a height of about 8 cm. The composition of mobile phase was optimized by using different mobile solvent of varying polarity. The HPTLC runs were in laboratory conditions of 25±5ºC and 50% relative humidity. After development the plate was withdrawn and dried .Spots were visualized in UV light (UV cabinet, CAMAG, Switzerland).

Quantification

Quercetin was quantified with CAMAG TLC scanner 3 equipped with Wincats software version 1.3.4 and computer under the following condition: Slit width 6x 0.45mm, wavelength 254nm UV (Deuterium lamp) absorption –reflection detection mode.

Preparation of Standard solution

10mg of standard quercetin were accurately weighed, quantitatively transferred into 10ml volumetric flask, dissolved in methanol and the volume was adjusted with the same solvent upto the mark.

Preparation of Sample solution

Air dried *A.nilotica* flowers (1g) were chopped into small pieces and exhaustively extracted using methanol (4x10ml) for 16-20 hrs. The solvent was evaporated under reduced pressure producing the crude extract (15% w/w).100mg of crude extract was transferred quantitatively into a10ml volumetric flask and dissolved in methanol adjusted to volume with methanol and shaken to mix thoroughly.

Calibration graph

2 -7µl of stock solution containing 2, 3,4,5,6 and 7µg were applied to HPTLC plate and plate was developed as above and scanned at 254nm. Calibration graph of quercetin was constructed by plotting concentration versus spot area of the compounds [13-15].

Recovery

For percent recovery, known concentrations of standards were added to a pre analyzed sample of *A.nilotica* flowers. The spiked samples were than analyzed by proposed HPTLC method. The experiment was conducted in duplicate.

Limit of detection (LOD) and Limit of quantification (LOQ)

Limit of Detection (LOD) and Limit of Quantification (LOQ) were experimentally verified by diluting known concentration of standard quercetin solutions. The results are shown in table 1.

Table 1. Method validation parameters for the estimation of Quercetin by the proposed method

Sl .No.	Parameter	Results
1	Linearity range	2000-7000ng $spot^{-1}$
2	Standard deviation(according to area)	0.61
3	Correlation coefficient	0.9999
4	Limit of detection	160ng
5	Limit of Quantification	915ng
6	Robustness	Robust
7	Recovery	102%
8	Specificity	Specific
9	Amount of Quercetin	11.5%

RESULT AND DISCUSSION

Phenolic compounds have been analyzed by various chromatographic techniques including high performance liquid chromatography (HPLC) , High speed counter current chromatography (HSCCC), Capillary zone electrophoresis (CE) and HPTLC, in various medicinal plants. None of the techniques have been fully applied to the analysis of phenolic compound in the

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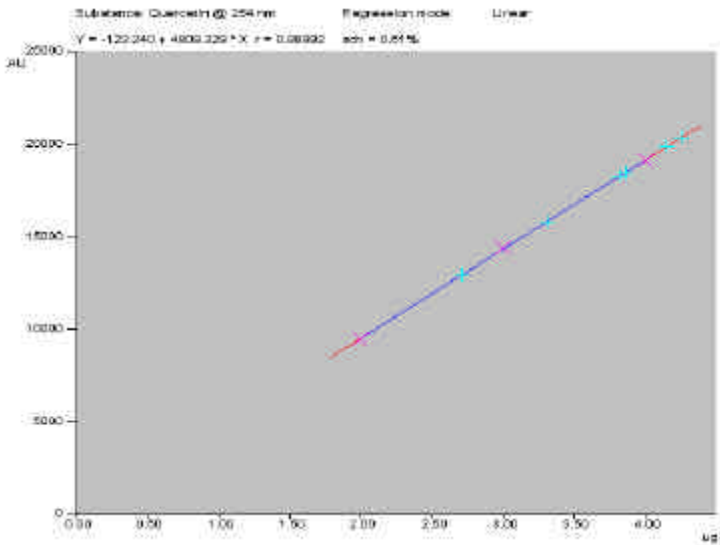

Fig 1: Calibration plot obtained by chromatography of marker compound. Regression via Area regression mode = Linear

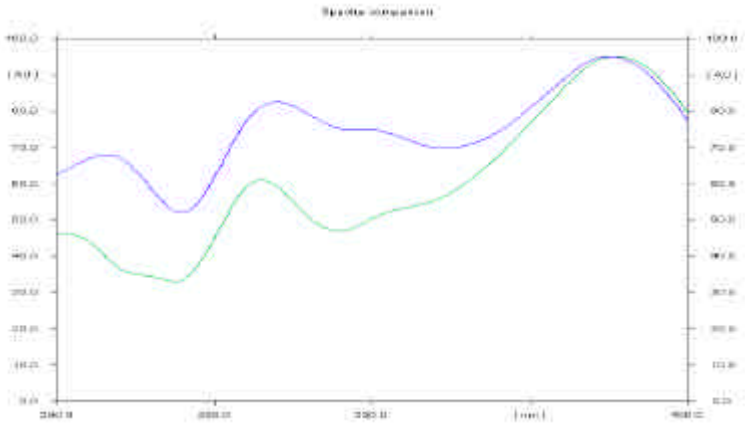

Fig 2: Super imposable spectra of Marker compound and sample

flower of *A.nilotica*. Moreover this simple and fast HPTLC method for quantitative determination of quercetin in methanolic extract of flowers of *A.nilotica* was developed for the first time.

Different composition of the mobile phase were tested and good resolution was achieved by using chloroform- methanol - water (7:3:0.5v/v/v) as mobile phase for quercetin with R_f 0.75 (Fig 3). The identified band of quercetin in the sample extract was confirmed by overlaying its UV absorption spectrum with that of standards using a CAMAG TLC scanner 3. The purity of quercetin bands in the sample extract was confirmed by comparing the absorption spectra's at start, middle and end position of the band (Fig 2). The calibration graph of quercetin was

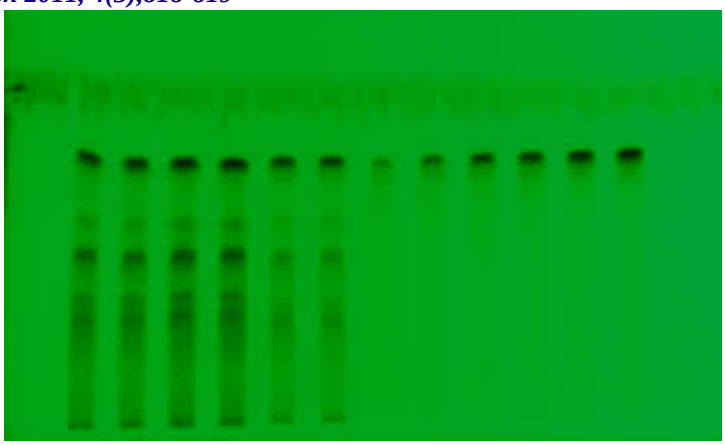

Fig 3: Image of developed plate

linear in the range of 2000ng to 7000ng (Fig 1) .Y= 123.240+4809.329*X. The average recovery of quercetin was 102%, lower limit of detection (LOD) obtained for quercetin was 160ng respectively with good linearties while the limit of quantification (LOQ) obtained were 915ng respectively. Quercetin in methanolic extract of A.nilotica was estimated by the proposed method.

CONCLUSION

The developed HPTLC method is an attractive alternative for the quantitative determination of quercetin in methanolic extract of flower of *A.nilotica* with regard to the simplicity, accuracy and selectivity. This method could be widely applied directly for routine analysis and quality assurance of related extracts and drugs.

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REFERENCES

1. Evans WC ,Trease and Evans Pharmacognosy ,14th Ed .Harcourt Brace and company, Asia PTE Ltd., 1996, pp.249
2. Haslam E J Nat prod 59; 1995, 205-215
3. Anonymous, Medicinal Plants of India, Vol.I, Indian Council of Medical Research, New Delhi, 1976, PP.6-8.
4. Anonymous The Wealth of India, Raw Materials Vol.I- CSIR, New Delhi, A,(Rev), 1985, PP.37.
5. Anonymous Phytochemical investigations of Certain Medicinal Plants Used in Ayurveda and Siddha, CCRAS, New Delhi, 1990, PP.187.
6. Anonymous Indian Medicinal Plants, Eds. Warrier, P.K. et al., Vol. 1, Orient Longman Ltd., Madras, 1994, PP.26.
7. Chopra, R.N. et al. Glossary of Indian Medicinal Plants, C.S.I.R., New Delhi, 1956, PP.2.
8. Sharma P.C, Yeine M.B, Dennis T.J, Database on Medicinal plants used in Ayurveda , Vol.1., Central council for Research in Ayurveda and Siddha , 2000, PP.57-64.
9. Chung CP, Park JB, Bae KH Planta Med 61: 1995, 150-153.
10. Cook NC, Samman S, J Nutr Bio-Chem 7: 1996, 66-76.
11. Formica JV, Regelson W Food Chem Toxic 33: 1995, 1061-1080.
12. Hanasaki Y, Ogawa S, Furkui S Free Rad Biol Med 16: 1994, 845-850.
13. ICH, Q2A Validation of analytical procedure: methodology in: Proceedings of the international conference on Harmonization, Geneva October 1994.
14. ICH, Q2B Validation of analytical procedure: methodology in: Proceedings of the international conference on Harmonization, Geneva March 1996.
15. ICH guidance on analytical method validation in proceedings of the international convention on Quality for the pharmaceutical industry, Torinto, Canada, September 2002.

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